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MECHANISM OF DNA TRANSLOCATION IN A REPLICATIVE HEXAMERIC HELICASE

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During DNA replication, two complementary DNA strands are separated and each becomes a template for the synthesis of a new complementary strand. Strand separation is mediated by a helicase enzyme, a molecular machine that uses the energy derived from ATP-hydrolysis to separate DNA strands while moving along the DNA. We determined a crystal structure of a viral replicative helicase bound to single-stranded DNA and nucleotide molecules at the ATP-binding sites. This structure demonstrates that a single strand of DNA passes through the hexamer channel and that the DNA-binding hairpins of each subunit collectively form a spiral staircase that sequentially tracks the DNA backbone. It also demonstrates a correlation between the height of each DNA-binding hairpin in the staircase and the ATP-binding configuration, suggesting a straightforward mechanism for DNA translocation.

Papillomaviruses are tumor viruses that cause benign and cancerous lesions in their host. Replication of papillomaviral DNA within a host cell requires the viral E1 protein, a multifunctional protein. E1 initially participates in recognizing a specific replication origin DNA sequence as a dimer with E2, another viral protein. Subsequently, further E1 molecules are assembled at the replication origin until two hexamers are established. These hexamers are the active helicases that operate bidirectionally in the replication of the viral DNA. In order to unwind DNA, helicases must separate the two strands while moving along, or translocating on the DNA. Based on structures of the DNA-binding domain of E1 bound to DNA that we determined a few years ago, we suggested a mechanism for DNA strand separation. However, the mechanism that couples the ATP cycle to DNA translocation has been unclear. The E1 hexameric helicase adopts a ring shape with a prominent central channel that has been presumed to encircle substrate DNA during the unwinding process, but the atomic details of this binding have been uncertain, including whether the ring encircles one or both strands of DNA during unwinding.

Authors (from left) Leemor Joshua-Tor and Eric Enemark

Our crystal structure of the E1 hexameric helicase bound to single-stranded DNA (**Figure 1**) demonstrates that only one strand of DNA passes through the central channel and reveals the details of the non-specific binding (**Figure 2**). The β-hairpins (DNA-binding hairpins) of each subunit sequentially track the sugar-phosphate backbone of the DNA in a one nucleotide per subunit increment. This configuration resembles a spiral staircase (**Figure 2**).

ATP-binding (and hydrolysis) sites are located at the subunit interfaces, and multiple configurations are observed within the hexamer. These have been assigned as ATP-type, ADP-type, and apo-type. The configuration of the site for a given subunit correlates with the relative height of its DNA-binding hairpin in the staircase arrangement. The subunits that adopt an ATP-type configuration place their hairpins at the top of the staircase while the hairpins of apo-type subunits occupy the bottom positions of the staircase. The hairpins of the ADP-type subunits are placed at intermediate positions.

A straightforward "coordinated escort" DNA-translocation mechanism is inferred from the staircased DNA-binding and its correlation with the configuration at the ATP-binding sites. Each DNA-binding hairpin maintains continuous contact with one unique nucleotide of ssDNA and migrates downward via ATP-hydrolysis and subsequent ADP-release at the subunit interfaces. ATP-hydrolysis

occurs between subunits located toward the top of the staircase, while ADP-release occurs between subunits located toward the bottom of the staircase. The hairpin at the bottom of the staircase releases its associated ssDNA phosphate to conclude its voyage through the hexameric channel. Upon binding a new ATP molecule, this subunit moves to the top of the staircase to pick up the next available ssDNA phosphate, initiating its escorted journey through the channel and repeating the process. For one full cycle of the hexamer, each subunit hydrolyzes one ATP molecule, releases one ADP molecule, and translocates one nucleotide of DNA through the interior channel. A full cycle, therefore, translocates 6 nucleotides with associated hydrolysis of 6 ATPs and release of 6 ADPs.

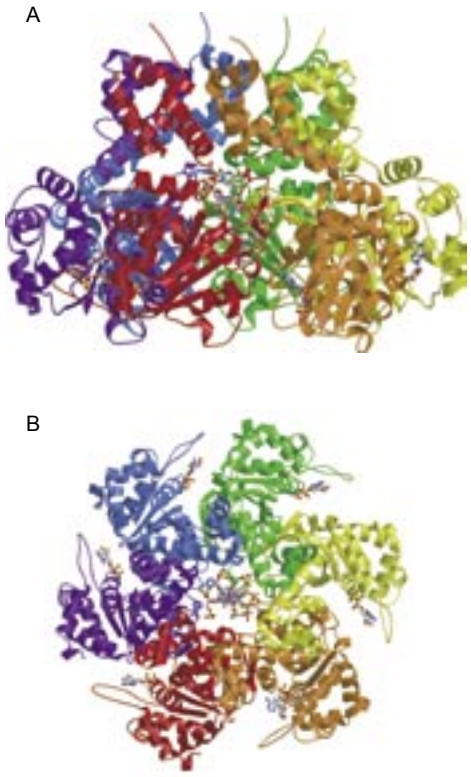

Figure 1. Views of the E1 hexamer parallel and perpendicular to the central channel with indidual subunits are color-coded. Single-stranded DNA is bound discretely within the chanel, and nucleotides are present at the subunit interfaces.

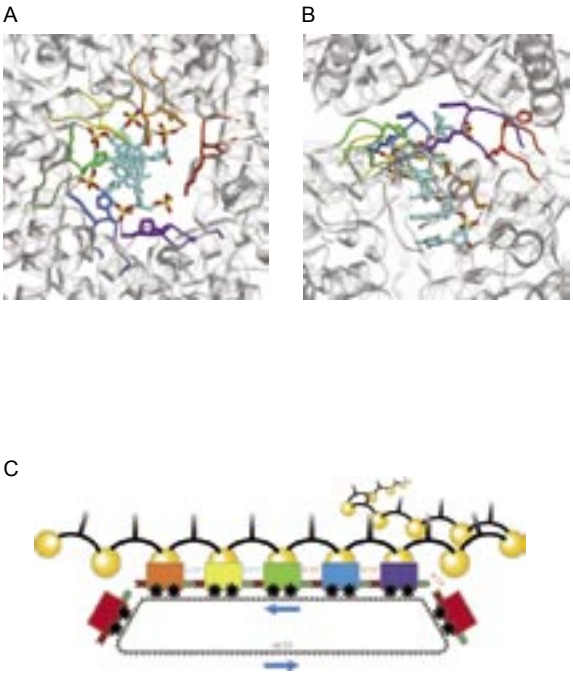

Figure 2. Details of DNA coordination viewed (A) parallel and (B) perpendicular to the hexamer channel and (C) a cartoon depicting the “coordinated escort” mechanism for DNA translocation.